33. (Amended) A method for identifying genes which carry a harmful allele or which are linked to a gene that carries a harmful allele, comprising:

a) identifying the inherited point mutations which are found in the genes or portions thereof of a population of young individuals, wherein the set of all inherited point mutations occurring at a frequency at about or above 5×10^{-5} can be identified, and determining the frequencies with which each point mutation occurs;

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b) identifying the set of inherited point mutations which are found in the genes or portions thereof of a population of aged individuals, and determining the frequency with which each point mutation occurs;

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c) comparing the frequency of each point mutation identified in a selected gene or portion thereof of the young population determined in a) with the frequency of the same point mutations identified in said selected gene of the aged population determined in b), wherein a significant decrease in the frequency of a point mutation in said selected gene of the aged population relative to said selected gene of the young population indicates that said selected gene carries a harmful allele or is linked to a gene that carries a harmful allele.

REMARKS

The title of the invention has been amended to be more clearly indicative of the invention to which the claims are directed. Claims 25 and 33 have been amended to further and more particularly define that which Applicants regard as their invention. Support is found throughout the Specification for these amendments. For example, the sensitivity of the methods of the claimed invention for detecting point mutations that occur at a frequency at or above 10^{-5} is described on page 17, lines 14-16, and at page 141, lines 11-13.

No new matter has been added by this amendment. Entry of this amendment is respectfully requested.

Rejection of Claims 25 and 33 Under 35 U.S.C. §102(b)

The Examiner has rejected Claims 25 and 33 under 35 U.S.C. §102(b) as being anticipated by Kervinen *et al.* (*Atherosclerosis*, 105:89-95, 1994).

The Examiner asserts that Kervinen *et al.* teach:

> identification of a harmful allele of apolipoprotein E (apo E) and apolipoprotein B (apo B) by determining the frequency of apo E and apo B polymorphisms in populations of young adults, middle-aged adults and nonagenarians. The frequencies of apo E ε4 allele and of apo B EcoRI R- allele were found to be significantly lower in nonagenarians than in young or middle-aged adults, indicating that the presence of these alleles suggests increased risk for coronary heart disease.
> Office Action, Page 2

Applicants have amended Claims 25 and 33 to indicate that point mutations can be detected down to a frequency of at least 10^{-5}. As Kervinen *et al.* do not teach such a detection method, the objection is obviated. Therefore, withdrawal of the rejection of Claims 25 and 33 under 35 U.S.C. §102(b) is respectfully requested.

Rejection of Claim 60 Under 35 U.S.C. §103(a)

The Examiner has rejected Claim 60 under 35 U.S.C. §102(b) as being obvious in view of Kervinen *et al.* (*Atherosclerosis*, 105:89-95, 1994) and Khrapko *et al.* (*Nucl. Acids Res.*, 22:364-369, 1994).

In addition to that which the Examiner asserts is taught by Kervinen *et al.* (see above), the Examiner further asserts that Khrapko *et al.* teach:

> (1) teach a method of determining point mutations in a DNA sample at a fraction of 10^{-6} or above using constant denaturant capillary electrophoresis (CDCE) combined with high-fidelity PCR....[and] (2) teach that prior to CDCE separation of the DNA fragments are boiled and reannealed, resulting in a mixture of homoduplexes and heteroduplexes, which are then separated based on the differences in their melting temperature in a CDCE capillary column.
> Office Action, pages 3-4.

The Examiner further states that "

> [i]t would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to have used the point mutation detection method of Khrapko et al. (1) and (2) in the method of mutation detection of Kervinen et al.. The motivation to do so, expressly provided by Khrapko et al., would have been that combining CDCE with high fidelity PCR permitted detection of low frequency mutations. Office Action, page 4

Applicants respectfully disagree (1) that it would have been *prima facie* obvious for one of ordinary skill in the art at the time of the invention to combine the teachings of Kervinen *et al.* and Khrapko *et al.*, and (2) that neither reference provides express or implied motivation to combine the teachings of these references.

Applicants further argue that, at the time of filing, methods contemplated in the art centered around analyzing known alleles or polymorphisms that were identified *en masse* by various genome and polymorphism sequencing projects. Since methods in the art at the time of filing were concerned with analyzing known alleles, it would not have been obvious for one of skill in the art to combine a method for analyzing allele frequencies in young and old populations with a method for detecting rare alleles. It was the Applicant that first attempted to overcome the problems associated with the methods available at the time of filing (see below) that led to the discovery of the disclosed invention.

The teachings of Kervinen *et al.* illustrate this state of the art; and they also illustrate the disadvantage of relying on already known alleles. Kervinen *et al.* analyze determine that a particular allele of apolipoprotein E is reduced in nonagenarians. This conclusion is based on a study group size of 89 individuals in the aged population, only four of whom are homozygous for the allele of interest. Even with such obvious problems of sampling error involved with this methodology, the art at the time of filing focused on merely analyzing the data readily available in public databases rather than detecting alleles de novo. Unfortunately, even with the great advances made by various sequencing projects in sequencing genomes, the information contained is not sufficient to eliminate the sampling error that is so readily apparent in the teachings of

Kervinen *et al.* In short, Kervinen *et al.* do not demonstrate that alleles for apoE or apoB are harmful by any reasonable biological or statistical standard.

By limiting the process to comparing allele frequencies of already known alleles, the teachings of Kervinen *et al.* will necessarily miss a vast majority of possible harmful alleles. As vast as the various genomic and SNP databases are, they represent only a minute fraction of the possible diversity contained in the population. As such, any method relying on known polymorphisms or alleles will necessarily be able to sample a minute fraction of possible alleles.

Additionally, the methods taught by Kervinen *et al.* to compare frequencies in different populations only allow for comparison of allele frequencies in a relatively small population (populations of about 100 were described).

Applicant's disclosure represents the first suggestion that the standard methods of analyzing known sequence data is insufficient for identifying harmful alleles by comparing allele frequencies in young and old populations. It is the unexpected finding that is possible to sample all alleles at any particular locus, not just the ones already known and available. It is the ability to sample all alleles, even alleles with allele frequencies as low as 10^{-6} in some cases, that gives the methods described in Applicant's disclosed invention a dramatic advantage over the methods known and contemplated in the art at the time of filing. For example, the teachings of Kervinen *et al.* describe detecting alleles with alleles frequencies in the range of 10^{-1}.

If, at the time, a method was available that overcame the problems associated with the known and commonly used methods in the art, it most certainly would have been employed. The fact that methods known in the art provided such limited information would indicate that improvements to the methods were needed and the lack of such improvements indicates that there were no obvious solutions to the problem. Thus, combining methods of comparing allele frequencies in young and old populations with a method for identifying rare alleles *de novo*, as the combination of the teachings of Kervinen *et al.* and Khrapko *et al.* would require, would not have been an obvious combination to one of skill in the art. The disclosed invention is the first suggestion that alleles can be detected without prior knowledge, at frequencies much lower than those known in the databases, and the frequencies of these alleles can be compared in large populations. Applicant is the first to suggest this combination in order to address significant

problems associated with methods practiced in the art at the time of filing. Therefore, the combination of cited references do not make obvious the claimed invention.

Moreover, there is no motivation to combine methods for detection of rare alleles with methods for comparing allele frequencies in young and old populations in order to identify harmful alleles. In the absence of an obvious motivation to combine in either cited reference, Applicant argues that the combination of the teachings of Khrapko *et al.* with the teachings of Kervinen *et al.* was not obvious at the time of filing. The lack of motivation to combine references further negates the Examiner's *prima facie* 103(a) rejection of Claim 60. Therefore, reconsideration and withdrawal of the rejection is respectfully requested.

Objection to Claims 26-28

Although no art is cited against Claims 26-28, they have been objected to as being dependent on the rejected Claim 25. Applicant believes that as a result of the above argument and amendment, Claim 25 will be allowed. Thus, no amendments to Claims 26-28 have been made.

CONCLUSION

In view of the above amendments and remarks, it is believed that all claims are in condition for allowance, and it is respectfully requested that the application be passed to issue. If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned at (978) 341-0036.

Respectfully submitted,

HAMILTON, BROOK, SMITH & REYNOLDS, P.C.

By Doreen M. Hogle

Doreen M. Hogle
Registration No. 36,361
Telephone: (978) 341-0036
Facsimile: (978) 341-0136

Concord, MA 01742-9133
Dated: July 18, 2002

MARKED UP VERSION OF AMENDMENTS

Specification Amendments Under 37 C.F.R. § 1.121(b)(1)(iii)

Replace the paragraph at page 1, line 1 with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

METHODS OF IDENTIFYING POINT MUTATIONS IN A GENOME THAT CAUSE OR ACCELERATE DISEASE

Claim Amendments Under 37 C.F.R. § 1.121(c)(1)(ii)

25. (Amended) A method for identifying genes which carry a harmful allele, comprising:

a) identifying the inherited point mutations which are found in the genes or portions thereof of a population of young individuals, wherein the set of all inherited point mutations occurring at a frequency at about or above 5×10^{-5} can be identified, and determining the frequencies with which each point mutation occurs;

b) identifying the set of inherited point mutations which are found in the genes or portions thereof of a population of aged individuals, and determining the frequency with which each point mutation occurs; and

c) comparing the frequency of each point mutation identified in a selected gene or portion thereof of the young population determined in a) with the frequency of the same point mutations identified in said selected gene of the aged population determined in b), wherein a significant decrease in the frequency of two or more point mutations in said selected gene of the aged population relative to said selected gene of the young population indicates that said selected gene carries a harmful allele.

33. (Amended) A method for identifying genes which carry a harmful allele or which are linked to a gene that carries a harmful allele, comprising:

a) identifying the inherited point mutations which are found in the genes or portions thereof of a population of young individuals, wherein the set of all inherited point mutations occurring at a frequency at about or above 5×10^{-5} can be identified, and determining the frequencies with which each point mutation occurs;

b) identifying the set of inherited point mutations which are found in the genes or portions thereof of a population of aged individuals, and determining the frequency with which each point mutation occurs;

c) comparing the frequency of each point mutation identified in a selected gene or portion thereof of the young population determined in a) with the frequency of the same point mutations identified in said selected gene of the aged population determined in b), wherein a significant decrease in the frequency of a point mutation in said selected gene of the aged population relative to said selected gene of the young population indicates that said selected gene carries a harmful allele or is linked to a gene that carries a harmful allele.